Supplementary Material

Synthesis of 1,1'-dicarboxycobaltocenium hexafluorophosphate (M_{ox})

Lithium methylcyclopentadienide (LiMeCp) and cobalt(II) acetylacetone (2:1 molar ratio) were added to THF in a dry ice/acetone bath under argon. The mixture was left to stir overnight at room temperature. The solvent was removed under vacuum, and the dark brown product $Co(II)(MeCp)_2$ was isolated by sublimation for 2 hours at 85°C. The product was mixed with $AgPF_6$ (1:1 molar ratio) and was left overnight to stir in CH_2Cl_2 under argon at room temperature. After filtering the solution to remove the silver, the solvent was removed under vacuum, leaving behind the yellow-green product $Co(III)(MeCp)_2PF_6$. This product was mixed with NaOH pellets and $KMnO_4$ (1:2:3 molar ratio, respectively). The mixture was refluxed in water for 4 hours, filtered hot through Celite, and an equimolar amount (c.f. $Co(III)(MeCp)_2PF_6$) of $NaPF_6$ was added. The solvent was then removed on a rotovap and the solid was washed repeatedly with acetone. The final product, $Co(III)(CpCOOH)_2PF_6$ (M_{ox}), was a bright yellow powder (λ_{max} = 410 nm).

Catalase Control

Analogous to the reaction conditions described in the main text, a thirty-minute reaction in air was conducted with 1 µM BM3 or hBM3, 1 mM lauric acid, and 1 mM reductant (M_{red}) in a final volume of 1 mL that also contained 2 µL of a $2.42x10^3$ Unit/µL solution of catalase. The reaction was conducted at room temperature. The sample was then prepared for analysis by GC/MS[1] (described in detail in the next section). A representative GC trace is shown in Figure S1.

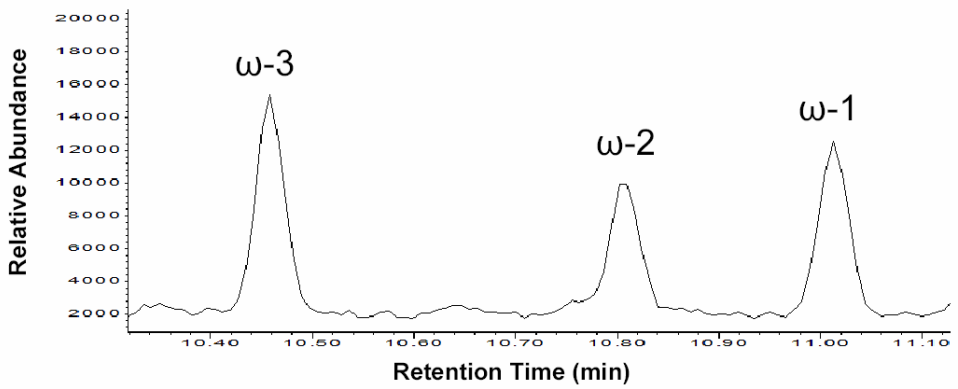

Figure S1: GC trace of a reaction containing BM3, M_{red}, lauric acid, and catalase. The sub-terminally hydroxylated products are labeled in the figure. The products were identified by MS.

The product profile was found to be similar for reactions with or without catalase. Negative controls (reactions lacking either enzyme, metallocene, or substrate) did not result in any observable products.

Bioelectrochemical Cell

All electrochemical reactions were carried out at room temperature. A mini-bioreactor was constructed using a stirred two-compartment cell, separating the working and Ag/AgCl reference electrodes from the Pt wire auxiliary with a glass frit. The working electrode was a 2 cm x 2 cm carbon cloth (ElectroChem, Inc.) that covered the bottom of the cell. A CH Instruments Electrochemical Workstation consisting of a potentiostat and software was used for the electrochemical reactions. The cell was first purged with a stream of argon, which was left on during the entire reaction. To the sample chamber, 2 mL of degassed buffer (170 mM KCl, 10 mM $MgCl_2$, 57 mM Tris-HCl pH 7.4) were added, as well as 2 mL of a degassed 5 mM solution of mediator (M_{ox} or Co(sep)) in the same buffer. 4 mL of degassed buffer were also added to the auxiliary chamber. A potential of -0.9 V was applied, and the mixture was allowed to stir for 6 minutes. At this point, 1 mL of a solution containing protein and 5 mM lauric acid were

added to the working chamber, and air was bubbled into the reaction using a Pasteur pipette at a rate of 1 bubble/second. Final concentrations and volumes were 1 mM lauric acid, 2 mM mediator, 1 μM BM3 or 1.5 μM hBM3, and 5 mL reaction volume.

Determination of Enzyme Activity in the Electrochemical System

0.5 mL aliquots were removed from the electrochemical cell at various time points and added to 3 drops of concentrated HCl to quench the reaction. The samples were then prepped for analysis using GC-FID as described[1]. Briefly, 5 μL of 50 mM 10-OH capric acid in DMSO (internal standard, described below) were added, and the reactions were then extracted three times with an equivolume of ethyl acetate. The ethyl acetate extracts were dried with sodium sulfate, followed by evaporation under vacuum. The samples were derivatized by adding 100 μL of a 1:1 mixture of pyridine:BSTFA, and then incubating at 80^{o}C for 20 minutes. 2 μL of these samples were then injected into the GC-FID with an HP-5 column.

A calibration curve was created to quantify the amount of product formed. Wild type BM3 sub-terminally hydroxylates lauric acid at the ω-1, ω-2, and ω-3 positions[2]. Since authentic standards for these compounds are not available, 12-OH-lauric acid was used to make the calibration curve with the assumption that the position of the hydroxyl group does not affect the FID response. An internal standard, 10-OH-capric acid, was used to aid quantification by adding a known amount to the samples, and then correlating the relative areas of 12-OH- to 10-OH-laurate to the amount of 12-OH-laurate initially present.

The reported rates in Table 1 were sustained for the initial 10 minutes of the reaction, after which a decrease in rate was observed (likely due to enzyme denaturation

from excessive bubbling of air into solution). Total turnover numbers were determined after 45 minutes of reaction, after which it was found that no further significant turnover occurred.

References

1. P.C. Cirino, F.H. Arnold, Adv. Synth. Catal. 344 (2002) 1-6.
2. N. Shirane, Z. Sui, J.A. Peterson, P.R. Ortiz de Montellano, Biochemistry 32 (1993) 13732-13741.